Reiko YOROI*: Gametophyte and apogamous embryo of *Crepidomanes latemarginale* from Isl. Ishigaki, Ryukyu**

鎧　礼子*: 石垣島産ミツデコケシダの配偶体と無配生殖により生じた胚**

Introduction: *Crepidomanes latemarginale* (Eaton) Copel. (Philip. Journ. Sci. **67**: 60, 1938) is a small epiphytic fern growing on wet rocks in valleys in the Ryukyus, Formosa to India.

Since Bower (1888), apogamous growth in the Hymenophyllaceae has been described on several species by a few workers. The embryological information in the family was very poor, including the recent work of Bierhorst (1975).

In this paper, an account will be given on this species as to the developmental morphology in the gametophyte, and the embryology in the sporophyte with the chromosomal data.

Materials and Methods: The fertile fronds of *Crepidomanes latemarginale* were collected in April 1973 by the author on Mt. Fukaiomoto-dake, Isl. Ishigaki, Okinawa Pref. Voucher specimens (Yoroi #5140) are deposited in the Herbaria of Tokyo Kyoiku University, Kyoto University, and University of Tokyo. The fertile fronds were washed with tap water, and the sporangia were removed from them to keep in the clean drag-papers, where the spores were shed. They were preserved in a refrigerator after being dried for several hours in the shade.

The spores were sown on the medium with inorganic nutrient in the Petri-dishes. The medium was Meyer's solution solidified with 0.8% agar. The dishes were placed in a culture box kept at 23-25°C under 12 hours' illumination a day with white fluorescent tubes at 300-1000 lux. The spores were also sown on sterilized soil in the small flower-pots, which were placed in a greenhouse for the long-term culture.

Apogamous embryos were fixed with Craf I (Sass, 1958), embedded in

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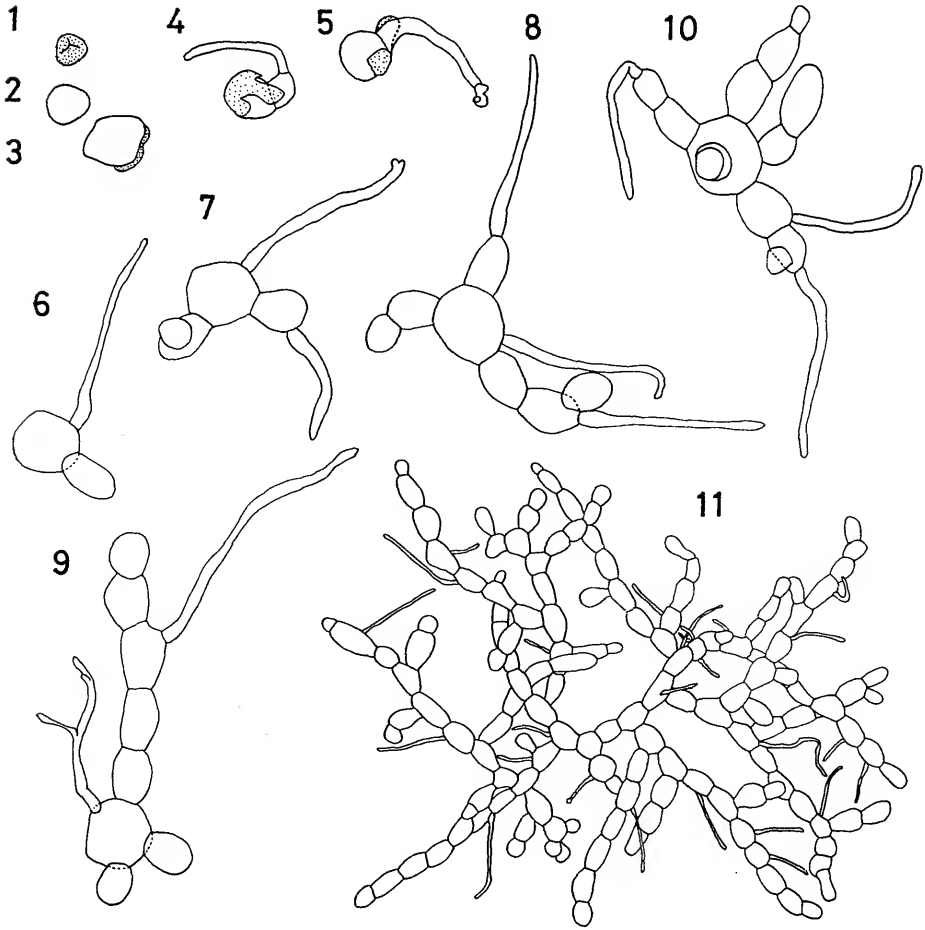

Fig. A. Germination of spores and young gametophytes. 1-10, ×86; 11, ×29. 1. Spore. 2-3. 16 days after sowing. 2. Basal cell. 3. Swollen basal cell with a spore coat. 4-5. 3 weeks. 4. First prothallial cell protruding. 5. First rhizoidal cell protruding. 6-8. One month. 6. Basal cell producing a prothallial cell and a rhizoidal cell. 7. Basal cell with two prothallial filaments and a rhizoid. 8. Basal cell with three filaments and a rhizoid. 9-10. One and a half month. 9. Basal cell with three filaments and a branched rhizoid. 10. Basal cell with five filaments. 11. Six and a half months. A prothallium with branched filaments and rhizoids.

paraffin, sectioned at 8 μm by the microtome, and stained with Delafield's hematoxylin. The somatic chromosome numbers were counted in the young frond-tips with the usual squash method as follows. The tips were immersed in 0.003 M 8-Hydroxyquinoline, fixed in Newcomer's solution, and hydrolized in 1N Hydrochloric acid and stained with acetocarmine. Photographs and camera-lucida drawings were made on the microtome sections of both gametophyte and sporophyte, including those of somatic chromosomes.

Observation: The spores are green, tetrahedral-globose in shape with three ridges of laesulae on the spore coat (Fig. A-1). The exine is thin and spiny. Usually, a sporangium contains 32 spores. The average diameter of the spores is about 50 μm.

When the exine splits at the laesulae as the spore swells, a cushion-like cell (basal cell) protrudes out of it (Fig. A-2). The cell has three obtuse corners directed to the former side-walls of the spore-coat and, in addition, a more obtuse corner directed to the former basal wall (Fig. A-3). The cell is chlorophyllous. The formation of the basal cell takes place within seven days after sowing. The fully swollen basal cell is two to three times as large as the original spore, but much smaller as compared with the other previously reported species of the family.

In about a dozen days after sowing, one of the four corners of the basal cell for the first time cuts off a prothallial cell (Fig. A-4). When the prothallial cell develops into a short prothallial filament with two or more cells by transverse wall formation, the second and the third prothallial cells begin to arise from the remaining three corners of the basal cell (Figs. A-6, 7, 8, 9, 10). At the corner directed to the former basal wall above mentioned, however, the development of the prothallial cell is much less frequent. Transformation to the rhizoidal cell is only recognized by the disappearance of the chloroplasts included in the distal cell of the prothallial filament. As to the filament directed to the basal wall, formation of the rhizoid is more frequent than the others (Fig. A-5). A new branch is often produced at the tip of the rhizoids without the wall-formation (Figs. A-7, 9). The prothallial cells often produce the lateral branch which may metamorphose to be the rhizoid.

Within six months, the profusely branched prothallium seems roughly to be a sphere of about 2 mm in diameter with the radially emitting fila-

ments and rhizoids in all directions, some of them being penetrated into the substratum (Fig. A-11). The cells in the filaments show variously shaped barrel-form and contain much less chloroplasts than the basal cell.

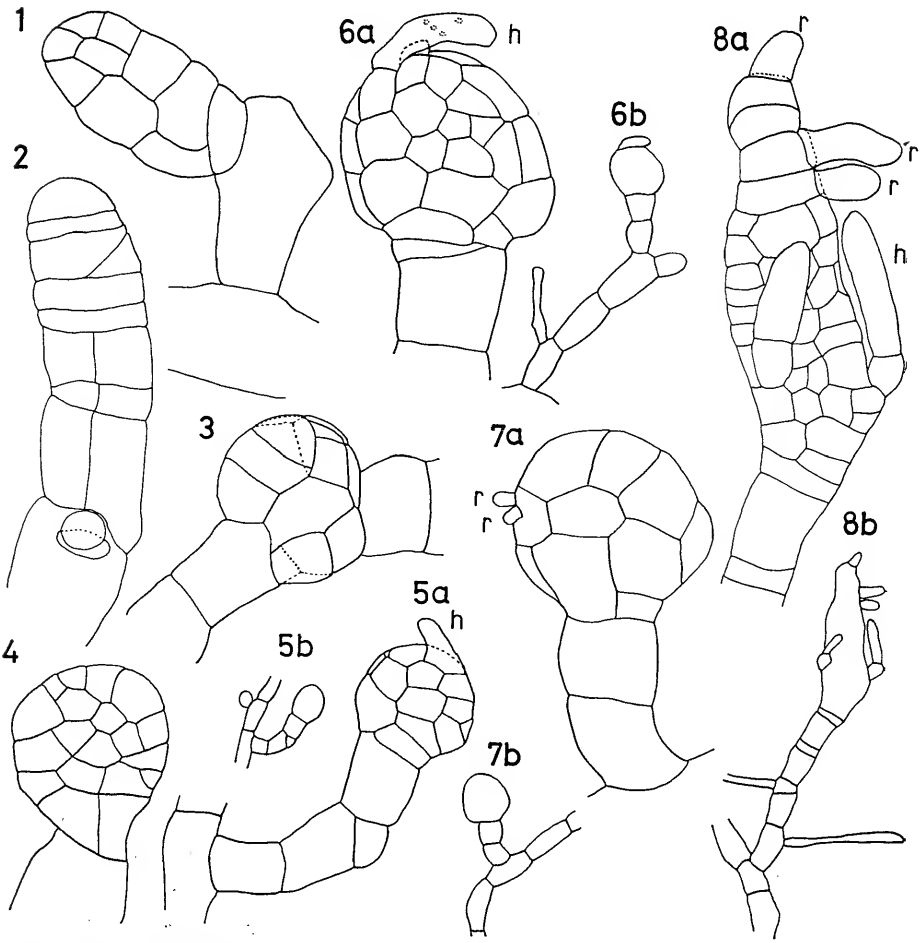

Fig. B. "Archegoniophores" and embryos. 1-4, 6a, 7a, ×240; 5a, 8a, ×165; 5b, 8b, ×41; 6b, 7b, ×60. 1-3. 15 months after sowing. 1. "Archegoniophore". 2. "Archegoniophore" with longitudinal cell divisions. 3. "Archegoniophore" on the midway of filament. 4. 16 months. Early stage of embryo. 5-8. Figures 5a-8a are magnified pictures of some parts of figures 5b-8b. 5. Embryo with a hair. 6. Embryo with hairs. 7. Embryo with two rhizoids. 8. Embryo with hairs and rhizoids. *h*, hair. *r*, rhizoid.

No longitudial cell division occurs in the filaments until the formation of embryos. As far as the author concerns, there are, on the filaments, no formation of antheridia, nor archegonia, nor gemmae within thirty-three months' cultivation.

In fifteen months' culture, the first oblique cell wall formation takes place on the prothallial filament. By the repeated oblique wall-formations, an apical cell is produced for the first time, as is shown in the formation of archegoniophores of *Crepidomanes bilabiatum* (N. and Bl.) Copel. (Stokey, 1948) and *Gonocormus minutus* (Bl.) v. d. B. (Yoroi, 1972). The apical cell functions for rather a short time, then loses its activity, resulting in the formation of a mass of cells of the same nature with the prothallial cells explained above (Figs. B-1, 2, 3). This mass of cells may be called archegoniophore, but it is slightly different from true archegoniophore in that no archegonium is actually formed on it. The author, hereafter, will use the term, "archegoniophore" with quatation mark as such. The "archegoniophore" is usually found at the distal end of the short lateral filament at the basal portion of the mother filament, or rarely at the midway on it. The "archegoniophore" gives rise apogamously to a young embryo. The author found several cases in which the intercalary longitudinal wall formation is observed as shown in Fig. B-2. The future development of this pattern is not worked out, but the resultant may be presumed to take the shape as shown in Fig. B-8. Figure B-4 shows external view of a younger embryo with the distal portion of the filament at its base. Figures C-1 and 2 show slightly oblique microtome cross sections of the young embryos with or without the small portion of the filament attached to it. The first which becomes discernible in longitudinal section of "archegoniophore" in its earliest development is a single apical cell, which is expected to develop into the first frond. The first frond is later accompanied by increasing number of the bicellular hairs (Figs. B-5, 6). Naturally, the first frond is formed at the distal end of the embryo, while the first rhizoid of the frond comes from near the proximal portion (Figs. B-7, C-3, D-1, 2). The rhizoid thus formed becomes coloured with pale brown and finally with brown. Three longitudinal serial sections in Fig. C-3 show a matured embryo with the apical cell of the first frond and procambial initials of the rhizome. The root is not recognized at all. Figures D-3 and 4 are external views of mature

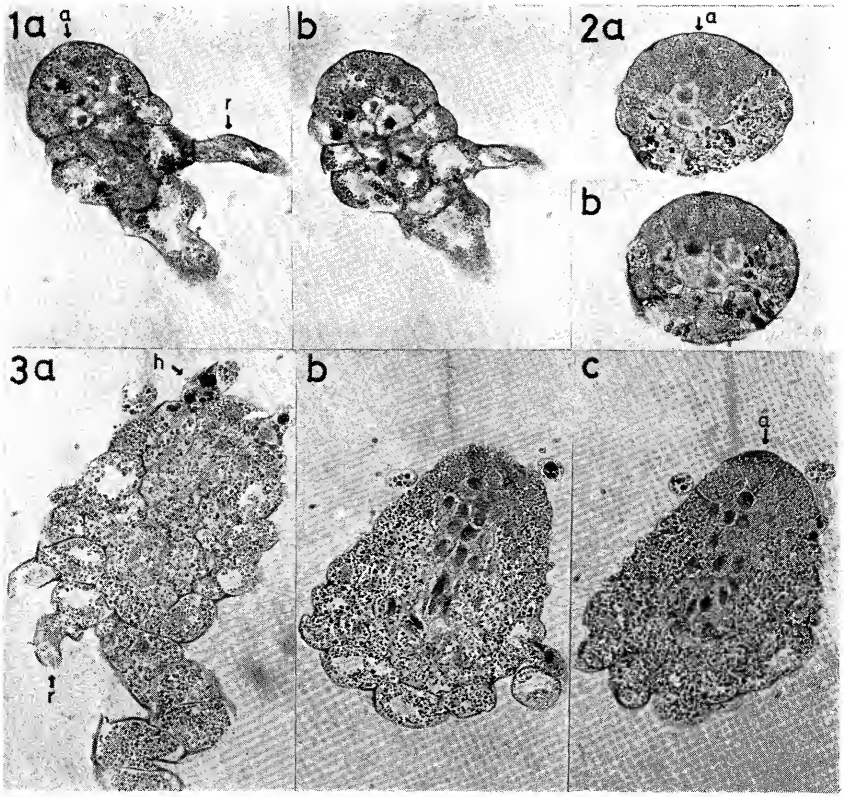

Fig. C. Sections of embryos. ×170. 1. Two sections of younger embryo with a rhizoid at its base. *r*, rhizoid. *a* (and arrow), apical cell. 2. Two sections of young embryo. 3. Three sections of embryo attached to the prothallial filament. *h*, hair.

embryos, showing the earlier stages of the rhizomes and the young fronds, with numerous rhizoids emitting from the whole length of the young plant. In figure D-6 the first frond attaining about 3 mm in length is presented, showing the distinct stipe and the lamina with the expanding wings of unicellular layer. The earlier stage of the first frond is shown in Fig. D-5. In rarer case, bifurcate hairs composed of three-cells are produced on the surface of the midrib (Fig. D-9). In this stage, numerous rhizoids arise from the lamina including the midrib. Occasionally, the tip of the midrib protrudes forwards to form a new rhizome which again develops into the lamina (Figs. D-7, 8).

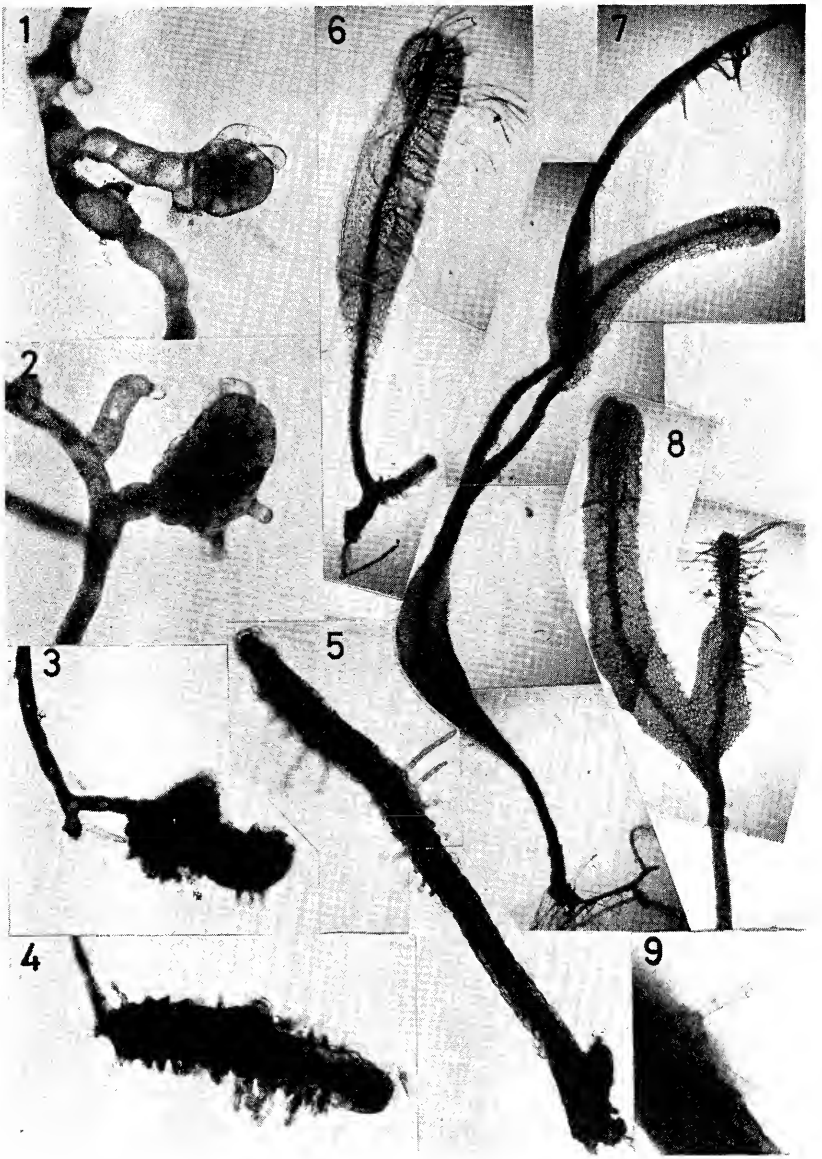

Fig. D. Embryos and the first fronds. 1-2, ×87; 3-4, ×44; 5, ×32; 6-8, ×18; 9, ×90. 1. Young embryo. 2. Old embryo. 3-4. Matured embryo. 5. Early stage of the first frond. 6. First frond with rhizoids on both lamina and midrib. 7. Tip of midrib protruding rhizomes and fronds. 8. Tip of midrib protruding a rhizome. 9. Bifurcate hair on the midrib.

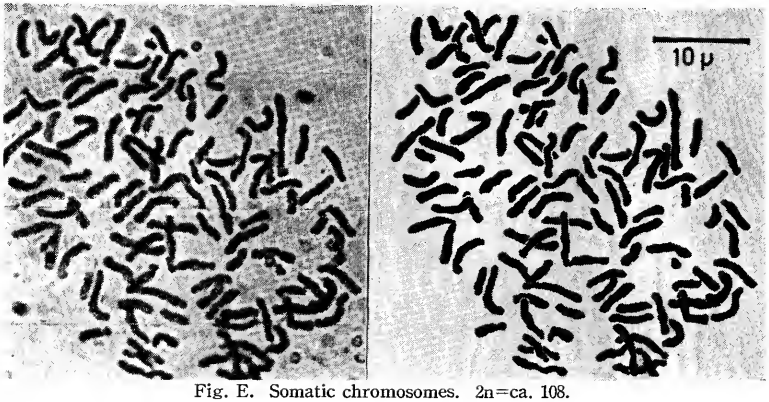

Fig. E. Somatic chromosomes. 2n=ca. 108.

The somatic chromosome number of approximately 2n=108 has been confirmed for this species (Fig. E).

Discussion: The type of spore germination in this species corresponds to "*Trichomanes*-type" according to Nayar and Kaur (1971), and to "Type I_p" and "I_r" of the author (1972). The gametophyte is of the type as usual in the other Trichomanoid ferns, but in this species no gemmae nor ribbon-like plates are discovered (Mettenius, 1864; Bower, 1888; Goebel, 1892; Stokey, 1940; Stone, 1958; Farrar and Wagner, 1968; Yoroi, 1972). If suppression occurs on the rhizoidal cell which may directly develop from any of the four corners of the basal cell, later development of the prothallial filaments are straightly directed, keeping the angle of 120 degree regularly between them. However, such a case is rarer. The usual case for this species is that the rhizoidal cell develops in earlier stage, and the aforesaid filaments take irregular disposition. The irregularity stated above are also observed in *Vandenboschia radicans* (Sw.) Copel. var. *orientale* (C. Chr.) H. Ito and *V. auriculata* (Bl.) Copel. (In Yoroi 1972, the first species is denoted simply as *V. radicans*). In the species concerned, however, the shape of cells and their inclusion are rather allied to those of Trichomanoid ferns, such as *Crepidomanes insigne* (v. d. B.) Fu and *Gonocormus minutus* (l. c.). In these species, the earlier development from the spore is quite regular. The irregularity and regularity mentioned above owes, the author considers, mainly to the specific nature of spores in those species, notwithstanding the influence from the laboratory condition.

The apogamous embryo has been commonly observed in numerous fern species (Farlow, 1874; Döpp, 1932; Whittier, 1962; Kanamori, 1972; etc.). But its successional development has only poorly described in the Hymenophyllaceae (Bower, 1888; Georgevitch, 1910; Stokey, 1948; Bierhorst, 1975). In *Crepidomanes latemarginale*, the young sporophyte developed from the "archegoniophore" was manifestly observed by the author, but the formation of sex organs has never been observed after about three years' culture. In pteridophyte, generally speaking, the apogamy takes place on the cushion, that is to say, multicellular layered portion of the plate-like gametophyte. In the Hymenophyllaceae, however, as there is no such portion, the archegoniophore plays a role in place of the cushion. In this species, as far as the author has observed, no archegonium is formed on the "archegoniophore". In *Vandenboschia auriculata*, Stokey (1948) reported the similar "archegoniophore", which in one case, produces archegonia and in another case does not. Bierhorst also reported an example of the apogamous development of the sporophyte in *Trichomanes* [s. lat.] *pinnatum* Hedw., in which archegoniophore produced archegonia. In his case, sporophyte arises de novo from the archegonial jacket. In the author's case, the problem is quite different. New shoot of sporophyte is produced directly from the mass of cells which constitutes the "archegoniophore". Consequently, the sporophyte develops much more rapidly than in the case of the other Trichomanoid ferns.

Considering the developmental morphology of both gametophyte and sporophyte, the average number of spores in the sporangium, and the basic chromosome number X=36 (Mitui 1968) in this genus, the plant collected by the author is considered to be an obligate apogamous triploid.

In the gross morphology, the herbarium specimens of *Crepidomanes latemarginale* (Eaton) Copel. collected by Tagawa and Iwatsuki #4830 on Isl. Ishigaki under the name of *C. intramarginale* (H. and G.) Copel. seem somewhat different from those of other localities (China and Thailand). The specimen collected by the author on the same island coincides quite well with Tagawa and Iwatsuki's.

Finally, the author takes note to the fact that in this species the organs such as the rhizome and the frond are less differentiated from each other, compared with those of the other species of the same family. This fact

might be attributed mainly to the environment in which this species lives; for example, extremely moist aerial or watery condition.

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琉球列島から台湾を経て印度まで分布するミツデコケシダの配偶体と胚発生を胞子（石垣島で採集）からの培養実験（2年9ヶ月）によって観察した。胞子の発芽様式は Nayar と Kaur の *Trichomanes*-type を示す。また著者の発表した（1972）I_p 型と I_r 型をあわせて示すが，初生仮根の発生が早いため，その後の発達が不規則になりがちである。前葉体は糸状体で，無性芽ならびに生殖器官の発達は培養期間中には観察されなかった。前葉体の基底細胞に近い側枝の細胞が塊状に発達すると，若い胞子体がその細胞群から無配生殖によって生じる。塊状の細胞群の発達は初期には造卵器床の発達に良く似ているが，造卵器形成が全く見られないことが特長的である。胞子

嚢1個あたりの胞子数は32個で，胞子体の体細胞染色体数は約 2n=108 であった。配偶体と胞子体の観察結果から，石垣島のミツデコケシダは3倍体の真性無配生殖種であると認めた。そして田川教授達および著者が採集した石垣島産の標本は，今まで採集された本種とは外部形態的に多少異なっている。また，この資料の若い胞子体では，根茎と葉との分化が明瞭でないことが観察された。詳細については今後の研究をまちたい。

□ 中井猛之進： **朝鮮森林植物編** I-XXII 附総索引 覆刻版，国書刊行会，東京 全10巻
NAKAI, T.: Flora Sylvatica Koreana, I-XXII with general index republished by Kokusho-kankokai, Tokyo, 1976, I. ¥95,000. 中井先生の朝鮮森林植物編は，先生の25年間にわたる労作であり，朝鮮総督府が世界に対して行った貢献の一つであって，その真価は日本国内よりも外地において甚だ高かった良書である。それが近来の古書覆刻の波に乗って再版されたことはまことに喜ばしいことであった。今その著を前にして，先生の研究の壮大なことに感じ入るのは，あながち小生一人ではないであろう。

覆刻に当っては，2-3巻をまとめて1巻とするなどして9巻にまとめ，さらに東京都立大学理学部牧野標本館の手になる，学名，和名及び朝鮮名の総索引1巻を加えて，全10巻としたのは，従来索引のないための不便を償って余りあるものであった。

各巻共に主要なる引用文献，朝鮮産その科の研究の歴史，朝鮮におけるその科の分布の概況，効用，分類と各種の図説という風に編集され，ことに最後のものが各巻の主要部を占め，かつは日羅両文から成り，また精巧な銅版による図版が添えられている。何しろ25年にわたる出版だから，研究も次第に高昇して，あとの方ほど科や属の区分，節の新設も目立ち，第21巻の如きは Rafflesiales 目の新設，Nandinaceae の独立，*Asiasarum* の設立など，内容は大変に発展している。第18巻のヤナギ科の花部構造と花の発達論もユニークな論議であって，これらが比較的見易くなったことは喜びにたえない。一，二気づいたことを添記すると，各巻は年次を異にして出版されたこと故，その出版年代を明記してほしかったこと，第12輯のホルトノキの図版 t. 17 の脱落は惜しい。 （前川文夫）

□ E. H. WALKER: **Flora of Okinawa and the southern Ryukyu Islands** pp. ix, 1159, plates 18, figs. 185, Smithsonian Institution Press, Aug. 1976, $36.75. Walker 博士が1951年に初めて沖縄本島を採集して以来，全精力をあげて努力されたこの大著が出版された。その仕事は園原，多和田，天野の諸氏との協力が始まり，著者自身の前駆的な小著の出版を経て，米本国側の公式なプロジェクトにのり，幾多の